1250-Plat
ATP Acts as Switch for Toggling Calreticulin Between its Lectin and Chaperone Function
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Calreticulin (CRT) is a lectin-binding chaperone that plays an important role in the assembly and folding of the major histocompatibility complex (MHC) class I proteins that present antigenic peptides on the cell surface and enable their recognition by T-cells. CRT binds to both specific oligosaccharide structures on MHC class I during their folding as well as a polypeptide part of MHC and thus plays a crucial role in stabilizing folding intermediates, preventing aggregation, and allowing the MHC protein to attain its native structure. Recent experiments have shown that the initial interaction between CRT and a monoglucosylated MHC protein is glycan-driven with glycan-independent interactions representing a second step in the chaperone cycle of CRT. Still, what factors trigger a switch from glycan-dependent to glycan-independent mode of interactions between CRT and its substrate is not well understood. Through computational investigations involving molecular dynamics simulations and structure based molecular docking, we show that ATP-binding serves as a switch for toggling CRT between two distinct modes of interactions. Specifically, the binding of ATP on CRT at the location that is distant from the glycan binding site leads to reduction in the affinity of CRT toward glycans and thus acts as a switch that induces a disengagement of a glycan from CRT, and induces exposure of hydrophobic regions on the surface of the globular domain allowing CRT to act as a chaperone through favoring interactions with the polypeptide component of the MHC. Further, employing community network analysis we have predicted residues that participate in allosteric signaling between ATP and glycan binding site of CRT. Modifying these residues has a large effect on the communication pathways in CRT consistent with experiments and support our model of ATP as a switch for CRT function.

1251-Plat
Allosteric Opening of the Polypeptide-Binding Site When an Hsp70 Binds ATP
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The 70 kD heat shock proteins (Hsp70s) are ubiquitous and highly conserved molecular chaperones essential for cellular protein folding and proteostasis. Each Hsp70 has two functional domains: a nucleotide-binding domain (NBD) that binds and hydrolyzes ATP, and a substrate-binding domain (SBD) that binds extended polypeptides. NBD and SBD interact little when in ADP; however, ATP binding allosterically couples the polypeptide- and ATP-binding sites. ATP binding promotes polypeptide release; polypeptide rebinding stimulates ATP hydrolysis. This allosteric coupling was poorly understood. To explore the molecular mechanism of this essential ATP-induced allosteric coupling, we solved a crystal structure of an intact Hsp70 from *E. coli* in complex with ATP at 1.96 Å resolution. NBD-ATP adopts a unique conformation, forming extensive interfaces with a radically changed SBD that has its α-helical lid displaced and the polypeptide-binding pocket of its β-subdomain flipped open. Our biochemical analysis inspired by this structure provides a long-sought mechanistic explanation of how ATP binding allosterically opens the polypeptide-binding site.

1252-Plat
Stability and Dynamics of Alpha Crystallin Oligomers Probed by FRET and FCS Reveal Persistent Oligomerization Under Dilute Conditions
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α-Crystallin is the major protein component of the human lens and plays an important role in the prevention of cataracts. α-Crystallin (αX) oligomers exist as a range of sizes varying from approximately 20-40 monomers. αX has two isoforms, αX-A and αX-B, which share high sequence similarity and define the common α-Crystallin fold found in many small heat shock proteins (sHSPs). αX-A and αX-B are hypothesized to play two important roles within the lens. First, they contribute significantly to the uniform, high protein density within the lens that enables it to cleanly focus light. Second, αX-A and αX-B both function as sHSPs that bind to misfolded proteins and prevent the formation of large, insoluble protein aggregates (the beginning of cataracts). Determining the mechanisms of αX chaperone function, oligomerization, and how the two are related will further the understanding of sHSPs in general and may lead to new preventions and treatments of cataracts. Several crystallographic studies of αX dimers have revealed a C-terminal strand exchange across a dimer interface. The dynamics of this C-terminus has been implicated in chaperone function and oligomer stability. Using fluorescence correlation spectroscopy (FCS) and intermolecular Förster resonance energy transfer (FRET) under both ensemble and single molecule conditions we investigate the stability of αX-B oligomers and their rates of subunit exchange. We present a method for disaggregating these high order oligomers under dilute conditions as well as describe conditions for generating kinetically trapped oligomers stable at nM concentrations.

1253-Plat
Enhanced Chaperone Clustering Facilitates Protein Folding in the Endoplasmic Reticulum of Yeast
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The chaperone BiP plays several roles in the endoplasmic reticulum (ER): translocation, protein folding, ER-associated degradation, and a modulating function in Ire1p-regulated ER stress. Experimental evidence has suggested the existence of BiP heterogeneity in the ER. A cooperative mechanism known as entropic pulling has been proposed to explain how the molecular interaction of multiple BiP molecules on unfolded proteins occurs. We have developed a model to explore the potential advantages of the binding of multiple BiP molecules in the facilitation of protein folding in the ER to explain the heterogeneity, and take advantage of entropic pulling.
Simulation scenarios were enacted to gauge the effectiveness of multiple chaperone binding in protein folding. Using two metrics: folding efficiency and chaperone cost, we determined that the single binding site model had a higher efficiency than multiple binding models, in the absence of cooperativity. Through entropic pulling, however, multiple chaperones do work in concert to facilitate the resolubilization and ultimate yield of folded proteins. Under a cooperative scenario, multiple binding models used fewer chaperones and enjoyed a higher folding efficiency than the single binding site model.
In conclusion, our in-silico experiments reveal that clusters of BiP molecules bound to unfolded proteins could enhance folding efficiency through cooperative action via entropic pulling.

Platform: Protein-Lipid Interactions II

1254-Plat
Molecular Mechanisms of High-Affinity Phosphoinositide Binding by the Tandem C2 Domains of Granuphilin/Slp-4
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Membrane-targeting proteins are crucial components of many cell signaling pathways, including the secretion of insulin. Granuphilin, also known as synaptotagmin-like protein 4, functions in tethering secretory vesicles to the plasma membrane prior to exocytosis. Granuphilin docks to insulin secretory vesicles through interaction of its N-terminal domain with vesicular Rab proteins; however, the mechanisms of its plasma membrane targeting and release are less clear. Granuphilin contains two C2 domains, C2A and C2B, that interact with the plasma membrane lipid phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P2]. Here we identify membrane-binding mechanisms, affinities, and kinetics of both granuphilin C2 domains using fluorescence spectroscopic techniques. Results indicate that both C2A and C2B bind anionic lipids in a Ca^{2+}-independent manner. The C2A domain binds liposomes containing a physiological mixture of lipids including 2% PI(4,5)P2 or PI(3,4,5)P3 with high affinity (apparent Kd of 2-5 nM), and binds nonspecifically with moderate affinity to anionic liposomes lacking phosphatidylinositol phosphate (PIPx) lipids. The C2B domain binds with sub-micromolar affinity to liposomes containing PI(4,5)P2 but does not have a measurable affinity for background anionic lipids. Both domains can be competed away from their target lipids by the soluble PIPx analogue inositol-(1,2,3,4,5,6)-hexakisphosphate (IP6), which is a positive regulator of insulin secretion. The results suggest potentially significant roles of granuphilin C2 domain-lipid interactions in the membrane docking and release of this protein during secretory signaling.

1255-Plat
NMR of Conditional Peripheral Membrane Proteins
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Conditional membrane proteins associate with membranes in response to binding specific ligands. We report the application of NMR techniques to gain insight into the structure, dynamics, and protein-membrane interactions of two fundamentally different conditional membrane modules: C1 and C2 domains, both

from the Protein Kinase Cα (PKCα), that are covalently linked to one another in the primary structure of the parent protein. Using NMR relaxation dispersion techniques, paramagnetic relaxation enhancement (PRE) experiments, and NMR-detected ligand binding studies, we demonstrate the role of conformational plasticity and initial membrane pre-association in modulating the affinity of the C1 domain to its natural cofactor, diacylglycerol. We show that the C2 domain employs a drastically different mechanism of membrane insertion that involves modulation of its electrostatic potential by divalent metal ions. The dynamics of loop and N- and C-terminal regions of C2 changes as a function of metal ligation state, suggesting a possible mechanism for propagating the information about the metal-binding event to other PKCα domains. In aggregate, our studies provide a view of conditional membrane domains as highly dynamic entities, in which conformational plasticity and synergistic action of cytosolic and membrane-embedded ligands define their nuanced signaling response. The functional and structural interplay between C1 and C2 domains will also be discussed in the context of the only two existing multi-domain structures of Protein Kinase C, one of which came from our laboratory.

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1256-Plat

The Role of Protein and Membrane Context in the Interaction of Polyglutamine Peptides with Lipid Membranes

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Huntington's disease is a dominant genetic neurodegenerative disorder associated with motor and cognitive decline, caused by a mutation in the polyglutamine (polyQ) region near the N-terminus of the huntingtin (htt) protein. Expansion of the polyQ region above 35-40 repeats results in the disease that is characterized by inclusion body aggregates of mutated protein. The polyQ expansion in htt flanked by a 17 amino acid N-terminal sequence (Nt17) and a proline-rich (polyP) region. To investigate the interaction between htt exon1 and lipid membranes, a combination of Langmuir trough techniques and vesicle permeability assays measuring calcein leakage were used to directly monitor the interaction of a variety of synthetic polyQ peptides with different combinations of flanking sequences (KK-Q_{35}-KK, KK-Q_{35}-P_{10}-KK, Nt17-Q_{35}-KK, and Nt_{17}-Q_{35}-P_{10}-KK) on total brain lipid extract (TBLE) model membranes. PolyQ peptides that lacked the Nt17 domain did not appreciably aggregate on or insert into lipid membranes. Nt17 facilitated the interaction of peptides with lipid surfaces while the polyP region enhanced this interaction. Our data suggests that the Nt17 domain plays a critical role in htt binding and aggregation on lipid membranes, and this lipid/htt interaction can be further modulated by the presence of the polyP domain. The addition of cholesterol to TBLE model membranes, to determine its role in htt-membrane interactions, resulted in reduced peptide insertion into lipid monolayers and decreased levels of induced vesicle permeability, though the effect does not scale linearly with cholesterol concentration. Results from parallel studies performed with ganglioside GM_1 and sphingomyelin to determine their role will also be presented.

1257-Plat

Association of α-Synuclein with Lipid Vesicles. Stopped-Flow Kinetics of Concerted Binding and Conformational Change

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Alpha-synuclein (AS), a 140aa intrinsically disordered protein, self-associates into oligomeric forms and aggregates into amyloid fibrils in Parkinson's disease. Certain mutations affect these processes and accelerate disease pathogenesis. The physiological roles of AS are a matter of speculation. Membrane binding is undoubtedly involved and the protein acquires α-helical structure in the process (1). We have studied the thermodynamics and kinetics of AS-membrane association utilizing vesicles (SUVs) of differing composition. Functionally neutral single cysteine mutants of AS were labeled with a polarity sensitive excited-state intramolecular proton transfer (ESIPT) probe (MFE). Double cysteine mutants were labeled with a FRET pair (Alexa Fluor488, Alexa Fluor568) at a series of selected positions in the primary sequence. Kinetic studies were conducted by stopped-flow, using 5-20 nM protein concentrations and increasing levels of SUVs (generally 20-200 μM) Signal changes indicative of membrane association were observed: increased intensity and shape change of dual band ESIPT emission, and altered FRET with the Alexa dyes. The analysis revealed a two-step reaction sequence in the time range <10 s. We attribute the first step to binding, and from the dependence on lipid concentration determined the second order rate constants and corresponding spectroscopic parameters. The second concentration independent step (1-10 s range) presumably arises from conformational changes in the protein (α-helix formation) and its accommodation to or perturbation of the lipid microenvironment (ESIPT dye).
Accompanying thermodynamic measurements led to estimates of dissociation constants as a function of membrane composition, charge, and shape (SUVs, LUVs). A new experimental protocol (*slopes*), implemented in a microplate reader, circumvented technical problems usually manifested in titrations of protein with lipid.

[1] Pfefferkorn C M, Jiang, Lee J C (2012). Biophysics of α-synuclein membrane interactions. *Biochim Biophys Acta***1818**:162-171.

1258-Plat

Fluorinated Aromatic Amino Acids Distinguish Cation-ϕ Interactions from Membrane Insertion

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Peripheral membrane proteins can be targeted to membranes via specific lipid interactions. Molecular dynamics (MD) simulations suggest that phosphatidylcholine (PC) cation / amino acid π complexes are important for the membrane binding of Bacillus thuringenisis phosphatidylinositol-specific phospholipase C (PI-PLC). This peripheral membrane protein specifically binds to PC-rich vesicles, and both mutagenesis and MD simulations suggest that choline cation / tyrosine π complexes provide most of the binding energy. The related Staphylococcus aureus PI-PLC has little affinity for PC, but introduction of two tyrosines at Asn-254 and His-258, mimicking the Bacillus enzyme, leads to PC specificity presumably due to cation-π interactions. However, there are not facile experimental methods for differentiating cation-π interactions from membrane insertion of aromatic side chains. By site-specific incorporation of pentafluorophenylalanine (F5-F) and difluorotyrosine (F2-Y) using the pEVOL system, we can distinguish these two types of interactions. Fluorinated amino acids are more hydrophobic, and are therefore likely to enhance binding by insertion. However, the altered electrostatics of the fluorinated aromatic ring should destabilize cation-π interactions. S. aureus F249(F5-F) has higher binding affinities than wildtype for small unilamellar vesicles (SUV) at all mole fractions of PC (XPC), and a ΔΔG relative to wildtype of -3.4 kJ/mol for binding to PC-rich SUVs(XPC=0.8) suggesting that the Phe inserts the membrane. In contrast, N254Y/H258(F2-Y) loses significant binding affinity for PC-rich vesicles compared to the parent N254Y/H258Y, with a ΔΔG relative to N254Y/H258Y of $+7.5$ kJ/mol at XPC=0.8, consistent with role of Tyr residues in cation-π complexes with choline headgroups. Thus fluorinated amino acids allow us to directly test how aromatic residues interact with membranes elucidating protein membrane interactions at molecular level, and provide both a direct test of MD simulations results and data that can be used to design membrane binding interfaces.

1259-Plat

In Vitro Reconstitution of Transcellular Tunnels Closure

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Several bacteria, such as Staphylococcus Aureus, are able to cross the endothelial barrier by inducing transcellular tunnels, called Transendothelial Macroapertures (TEM), in endothelial cells. The closure of these TEMs is critical to prevent endothelial permeability and cell death. Several proteins have been identified to play a role in this process. In particular, the I-BAR domain proteins MIM and ABBA have been shown to accumulate at the edge of the aperture shortly after the opening event. They subsequently recruit actin, followed by actin-rich membrane wave extension over the aperture. Interestingly, the related protein IRSp53, that unlike MIM and ABBA proteins does not have amphipathic alpha-helices on its I-BAR domain, has not been found at the edge of the TEM.
The details of this mechanism remain unknown. Our objective is to characterize the physics underlying the first step in TEM closure. Our hypothesis is that MIM and ABBA have the ability to recognize the newly negatively-curved membrane at the edge of the TEM through their I-BAR domain. We use a minimal system where the protein is encapsulated in a giant unilamellar vesicle and can interact with the negatively-curved inner surface of a membrane tube that has been pulled out of the vesicle. We study protein-membrane interactions through fluorescence (confocal microscopy) and force (optical tweezers) measurements. By combining the two types of measurement, we quantify the affinity of the proteins for curved interfaces, ranging in radii of curvature from 10 to 100 nm, as well as their potential mechanical effect on the membrane. Our results show an original behavior where ABBA and IRSp53 are maximally enriched in membrane tubes of specific radii of curvature.